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(54) **Protein Fraction for the Cosmetic and Dermatological Care of the Skin**

(57) The protein fraction from leguminosae seeds, which is characterized by at least one band in the polyacrylamide gel electrophoresis procedure with Na-dodecylsulfate, and by relative molecular masses from 3,000 to 30,000 g/mole, and by a total nitrogen content of 14 to 20% and by an amino nitrogen content of 1 to 2% relative to the protein content and by further parameters as defined in claim 1, is suitable for skin care and for the treatment of inflammatory diseases of the skin. In this regard, the protein fraction is generally used in the form of an active substance concentrate or, for example, as a creme, lotion, emulsion, gel, face mask, powder or plaster.

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The role of the skin, as an organ that envelops the organism, comprises sealing and mediating functions relative to the environment. Various biochemical and biophysical systems serve for maintaining the integrity of this exposed organ: for example, an immune system protects the skin from damage due to pathogenic microorganisms; the melanin-forming system regulates the pigmentation and protects the skin from radiation injuries; a lipid system produces lipid micelles that limit transdermal water loss; and a regulated keratin synthesis process provides the mechanically resistant horny layer. The designated systems are based on complex chemical processes whose course is sustained by enzymes and regulated by enzyme inhibitors. Even a slight inhibition or disinhibition of these biochemical systems manifests itself by detectable changes in the skin. However, the visible and perceptible state of the skin is considered to be a measure of beauty, health and youth; its maintenance constitutes a general objective of skin care cosmetics.

The moisture content of the skin plays a decisive role in its appearance, elasticity and perceptible texture. An extensible, elastic, delineating membrane, namely the stratum conjunctum that is located between the horny layer and the living epidermis, prevents the transdermal loss of water in healthy skin. This permeability barrier comprises lipids, proteins and carbohydrates, and it can therefore be damaged by extremely widely differing influences. For example, washing with surfactants can extract lipids and thereby increase the permeability of the stratum conjunctum with respect to water; ultraviolet irradiation can catalyze cross-linking or the cleavage of proteins, proteoglycans and polysaccharides thereby producing a decrease in elasticity and an increased susceptibility of the stratum conjunctum to injuries; bacterial enzymes can catalyze the degradation of the proteins and carbohydrates of the stratum conjunctum and, in the case of inflammatory processes and immune reactions, endogenous enzymes that are mobilized to an excessive extent, such as e.g. the tryptases, elastases and cathepsins, can attack the skin and, in particular, its permeability barrier.

Various cosmetic skin care products have been developed with the aim of increasing the moisture content of the skin and thereby improving its condition. A review of hydrating cosmetic active substances can be found in the publication by: S. D. Randazzo and P. Morganti, J. Appl. Cosmetol. 8, 93-102 (1990). Use is made of hydrophilic, hygroscopic substances, such as e.g. glycerine, sorbitol, various sugars, and protein hydrolysates. These substances are all readily washed away, however, and quite a lot of them leave behind an unpleasant, sticky feeling on the surface of the skin and, in the case of particularly marked hygroscopic properties, other substances can even dry out the horny layer. Use is also made of water-in-oil emulsions in order to supply the skin with moisture to an increased extent and to maintain this condition. However, these leave behind a tightly sealing layer of fat on the skin surface, and they cause water retention, an unpleasant swelling of the horny layer and, in the long term, disruptions, which can be repaired only with difficulty, of the lipid composition of the stratum conjunctum. Skin care products, which contain glycolipids and phospholipids from bovine brain in order to fortify the permeability barrier, bring about a measurably higher moisture content of the skin of test persons and they do not thereby produce the undesired, impermeable fatty film on the horny layer. Nevertheless, since lipids from bovine brain could be potential carriers of the pathogenic agent in bovine spongiform encephalopathy (BSE, “mad cow disease”), there is only a very limited demand for products of this type even when they are manufactured in accordance with processes that rule out this pathogenic agent with certainty.

The hydrating action of various plant lipids and proteins has been investigated using test persons in order, if possible, to find alternatives to active substances of bovine origin. It has now been found that locally applied proteins, which had been extracted from leguminosae seeds, increase the moisture content of the skin particularly markedly, whereby this effect is especially persistent in comparison to known hydrating agents. In some test persons, however, it was also found, surprisingly, that protein preparations from leguminosae seeds soothed itchiness at the locations of insect bites and, in test persons suffering from psoriasis, they clearly reduced the itchiness, skin redness and desquamation at the lesion sites. In a subsequent systematic investigation of the inflammation inhibiting action of proteins from legurninosae seeds, moreover, it was found that these substances more rapidly reduce local skin redness, which had been produced in test persons by trichloroethylene, compared to an analogous preparation without leguminosae proteins, whereby the inflammation inhibiting action that was observed was approximately as great as that of a control preparation that contained flumethasone. It was also found that the elasticity of skin sites, which had been treated with proteins from leguminosae seeds, was increased to a measurable extent. Furthermore, it was found that the designated proteins from leguminosae seeds inhibit proteinases such as e.g. tryptase, PMN-elastase, fibroblast-elastase and trypsin.

Hence the present invention pertains to a protein fraction, and to active substance concentrates that contain this protein fraction, and to preparations for skin care and/or for treating inflammatory skin diseases, whereby these preparations contain at least one protein that is obtainable from leguminosae seeds, or a protein fraction, that has

an inflammation inhibiting action, a hydrating action, a skin elasticity increasing action, and a proteinase inhibiting action.

The following, for example, are suitable as the leguminosae seeds for obtaining the proteins of the present invention: bean species, such as e.g. Phaseolus angularis, Phaseolus lunatus, Phaseolus aureus, Phaseolus vulgaris, Phaseolus coccineus, and Phaseolus limensis; pea species, such as e.g. Lathyrus odoratus; the soybeans Glycine max and Glycine hispida; the peanut Arachis hypogaea as well as the seeds of tropical leguminous plants of the Cajanus, Dolichus, Vigna and Vicia genus.

The protein fractions in accordance with the invention can be obtained from the designated leguminosae seeds by grinding the dried seeds, extracting the flour, which is thereby obtained, using an organic solvent or solvent mixture, drying and extracting the flour, which is degreased in this way, with water or an aqueous electrolyte solution at a pH value of 2 to 10, and preferably at pH 5 to 6, adjusting the extract to pH 5 to 7, evaporatively concentrating under vacuum, obtaining a clear material by centrifuging or filtering the concentrate with an addition being made of a filter aid such as e.g. kieselguhr, separating the proteins therefrom either by means of precipitation via a salt, e.g. ammonium sulfate at 30 to 80 % saturation, or by means of precipitation with an organic, water miscible solvent, such as e.g. ethanol, at a concentration of 60 to 90 %, collecting [the aforementioned protein fractions] by filtration or centrifugation and, finally, either drying them directly in a vacuum or first removing salts by means of dialysis, gel filtration or ultrafiltration, and then lyophilizing them. A protein fraction is obtained in the form of a dry substance that exhibits at least one band in the polyacrylamide gel electrophoresis procedure in the presence of sodium dodecylsulfate (Phastsystem, Pharmacia Biosystems, Uppsala, S [Sweden], whereby the electrophoretic mobility of the band permits relative molecular masses of 3,000 to 30,000 g/mole to be inferred. The protein fraction that is obtained exhibits a total nitrogen content of 14 to 20 % and an amino-nitrogen content of 1 to 2 %, based in each case on the protein content, and it is soluble in water and aqueous electrolyte solutions and it is insoluble in ethanol and acetone; this protein fraction exhibits marked precipitation in an aqueous solution following the addition of trichloroacetic acid, sulfosalicylic acid, picric acid or benzethonium chloride. In addition, the protein fraction inhibits proteinases and, for example, it exhibits an I_{50} value of less than 10 µg (based on the dry substance) per mL of test mixture when measured using trypsin, and an I_{50} value of less than 100 µg (based on the dry substance) per mL when measured using PMN-elastase, and an I_{50} value of less than 200 mg (based on the dry substance) per mL of test mixture when measured using tryptase, and an I_{50} value of less than 350 mg (based on the dry substance) per mL of test mixture when measured using fibroblast-elastase. When incorporated into suitable cosmetic or dermatological vehicles in concentrations of 0.1 to 2 % (calculated as the dry substance), the protein fraction that is obtained exerts the following effects on the skin of human test persons: a hydrating effect, a calming effect on itchiness, an inflammation inhibiting effect, and a skin elasticity increasing effect.

In regard to their dermatological and cosmetic application, the substances in accordance with the invention are expediently used in a skin friendly vehicle, e.g. in the form of a creme, lotion, gel, face mask, powder or plaster. With the exception of the plasters, the finished preparations generally contain 0.01 to 5 % by mass, and preferably 0.1 to 2 % by mass, of the active substance in the dry substance form. In contrast to this, plasters for the transdermal application of a preparation in accordance with the invention can contain up to 90 % by mass of the active substance. Active substance concentrates can be manufactured from the protein fractions in order to facilitate the formulation of liquid or semi-solid preparations, whereby these active substance concentrates are stable and readily capable of being administered and they have an active substance content of 1 to 15 % by mass, and preferably approximately 7.5 % by mass, in dry substance form, and whereby their manufacture takes place by allowing the protein fractions in accordance with the invention to dissolve in water and then providing them with an addition of a water soluble preserving agent, such as e.g. methyl p-hydroxybenzoate, in order to avert microbial growth, and with an addition of a polyhydric alcohol, such as e.g. ethylene glycol or propylene glycol, for protein stabilization purposes, and with an addition of a non ionogenic or amphoteric surfactant, such as e.g. polysorbate 80, octoxynol, cocoamphoglycinate or cocoamidopropylbetaine, in order to suppress any hydrophobic interaction and the phenomenon of protein flocculation that is associated therewith.

The trichloroethylene erythema test in accordance with the work of H. Friderich (Ärtzliche Forschung 20, 549-552, 1966), for example, is suitable for determining the inflammation inhibiting action of the preparations in accordance with the invention, and a reflectance [typo] colorimeter, such as e.g. the CR-300 Chroma-Meter from the Minolta Camera Co. firm in Osaka, Japan, for example, is suitable for the photometric evaluation of the test.

The measurement of skin elasticity can take place by applying suction to the skin of a test person by means of a hollow probe at a defined negative pressure and measuring the depth of penetration of the skin into the probe and subsequently normalizing the pressure, by admitting air, and then once again measuring the depth of penetration of the equilibrated skin into the probe. The speed and degree of restoration of the skin, which had been extended

as a result of the negative pressure, constitute a measure of the elasticity of the skin. The "Cutometer" apparatus from the Courage & Khazaka electronic GmbH firm in Cologne, D [Germany] is very suitable for measuring the elasticity of the skin in accordance with this principle, whereby this apparatus has been registered and it is equipped with a microprocessor. A determination of the degree of moisture of the skin can take place using the "Corneometer" electronic meter (Courage & Khazaka). A knowledge of the differing dielectric constants of water and the other skin components forms the underlying basis of this measurement principle.

In order to determine the in vitro proteinase inhibitor action of the preparations in accordance with the invention, the enzyme that is to be inhibited is incubated with the inhibitor or, respectively, a placebo over a defined period of time, and then it is mixed with a chromogenic proteinase substrate and the liberation as a function of time of p-nitroaniline, which is catalyzed by the residual non inhibited enzyme, is measured photometrically at a wavelength of 405 nm in the form of DA_{405}/t. The difference between the DA/t value for the reference and the DA/t value for the sample permits one to calculate the I_{50} value as the quantity of the preparation that inhibits the enzyme to the extent of 50 % under the defined conditions. The following are suitable as chromogenic substrates for PMN elastase and fibroblast elastase: MeOSuc-Ala-Ala-Pro-Val-pNA; the following is suitable as a chromogenic substrate for trypsin: Bz-Val-Gly-Arg-pNA; and the following is suitable as a chromogenic substrate for tryptase: Tos-Gly-Pro-Arg-pNA; (a comprehensive description of the methods for the determination of proteinases and the inhibitors thereof by means of chromogenic substrates can be found in the publication by I. Witt, Eur. J. Clin. Chem. Clin. Biol. 29, 355-374, 1991).

The proteinase inhibitor action can also be demonstrated in the lysate of cultivated fibroblasts. The determination takes place with a chromogenic proteinase substrate as described above.

In order to demonstrate elastase inhibition in an ex-vivo system, elastin rich tissue, such as e.g. a tendon, can be damaged in an organ bath by means of an addition of PMN elastase. This can be prevented by the simultaneous addition of the preparation in accordance with the invention.

Example 1

Manufacture of an Inflammation Inhibiting Protein Fraction from Soybeans

100 g of dried soybeans were ground, stirred twice over a period of 3 hours on each occasion with 300 mL of octane on each occasion, filtered through a suction filter, thoroughly pressed out and then the material, which had had been degreased in this way, was dried in a vacuum. The dried material was suspended in 1.35 L of demineralized water, which contained 0.5 % of the bacteriostat Phenonip^{R}, and the pH of the suspension was adjusted to 2.5 to 3 with hydrochloric acid; the mixture was then stirred for 2 hours at 20 °C and subsequently centrifuged. The opalescent supernatant liquor was adjusted to pH 5.2, evaporatively concentrated to 200 mL in a vacuum at 40 to 50 °C, mixed with 400 mL of saturated ammonium sulfate solution and then allowed to stand for 15 hours at room temperature. The protein precipitates were separated by means of centrifugation, made into a slurry in 100 mL of aqueous Phenonip solution 0.58 [sic; having a concentration of 0.5 %?], dialyzed overnight using 2 L of Phenonip water [sic; solution?], freed from separated material by means of filtration through an asbestos free filter layer and then, finally, lyophilized. A protein fraction was obtained that exhibited 4 bands in the polyacrylamide gel electrophoresis procedure in the presence of sodium dodecylsulfate, whereby the electrophoretic mobility of these bands permitted relative molecular masses of 3,000 to 30,000 g/mole to be inferred. The protein fraction that was obtained exhibited a total nitrogen content of 15.2 % and an amino-nitrogen content of 1.4 %, based in each case on the protein content, and it was soluble in water and aqueous electrolyte solutions and it was insoluble in ethanol and acetone; this protein fraction exhibited marked precipitation in aqueous solution following the addition of trichloroacetic acid, sulfosalicylic acid, picric acid or benzethonium chloride, and, when measured using trypsin, it exhibited an I_{50} value of 3 µg (based on the dry substance) per mL of test mixture and, when measured using PMN elastase, and an I_{50} value of 68 µg (based on the dry substance) per mL. When incorporated into a cosmetic lotion in a concentrations [sic; a concentration] of 0.75 %, the protein fraction that was obtained exerted the following effects when applied to the skin of human test persons: a hydrating effect, a calming effect on itchiness, an inflammation inhibiting effect, and a skin elasticity increasing effect.

Example 2

Manufacture of an Inflammation Inhibiting Protein Fraction from Lima Beans

100 g of dried lima beans (Phaseolus lunatus) were ground, stirred over a period of 1 hour with 500 mL of 95 % by volume ethanol and filtered through a suction filter; the moist residue was stirred twice over a period of 1 hour on each occasion with 500 mL of 1 N acetic acid on each occasion, and once again filtered through a suction filter. The combined acetic acid extracts were evaporatively concentrated to 100 mL in a vacuum, mixed with 50 % by

volume ethanol, and the precipitated polysaccharides were removed by means of filtration; the active proteins were precipitated from the filtrate by increasing the ethanol concentration to 80 % and then the active proteins were collected on a suction filter and dried in a vacuum. A protein fraction was obtained that exhibited 5 bands in the polyacrylamide gel electrophoresis procedure in the presence of sodium dodecylsulfate, whereby the electrophoretic mobility of these bands permitted relative molecular masses between 3,000 and 30,000 g/mole to be inferred. The protein fraction that was obtained exhibited a total nitrogen content of 14.9 % and an amino-nitrogen content of 1.3 %, based in each case on the protein content, and it was soluble in water and aqueous electrolyte solutions and it was insoluble in ethanol and acetone; this protein fraction exhibited marked precipitation in aqueous solution following the addition of trichloroacetic acid, sulfosalicylic acid or picric acid, and it exhibited an I_{50} value of 5 µg (based on the dry substance) per mL of test mixture when measured using trypsin, and an I_{50} value of 89 µg (based on the dry substance) per mL when measured using PMN-elastase. When incorporated into a cosmetic lotion in a concentrations [sic; a concentration] of 1.5 %, the protein fraction that was obtained exerted the following effects when applied to the skin of human test persons: a hydrating effect, a calming effect on itchiness, an inflammation inhibiting effect, and a skin elasticity increasing effect.

Example 3

Manufacture of a Stable Active Substance Concentrate

20 g of propylene glycol, 2 g of Tween 80 and 0.2 g of methyl p-hydroxybenzoate were dissolved in 60 mL of distilled water with heating to 70 °C. After cooling to 30 °C, 7.5 g of the protein fraction that had been manufactured in accordance with example 1 were dissolved in this solution, and the pH value was adjusted to 7, and then the volume was made up to 100 mL with distilled water.

Example 4

Determination of the Inflammation Inhibiting Action of a Soy Protein Fraction

Nine parts by volume of the commercial, skin-compatible emulsion "Excipial U Lotio" (Spirig AG, Egerkingen, CH [Switzerland]), which is free from active substances, were mixed in each case with one part by volume of distilled water (placebo) or [one part by volume] of the sample, which was manufactured in accordance with example 1 and which had been pre-dissolved in distilled water at a concentration of 75 mg per mL, and then homogenized. The skin of the forearm of two test persons was subjected to irritation for 5 minutes at four locations by means of 150 µL of trichloroethylene that had, in each case, been applied to patch test plasters. After this, two of the irritated skin regions were each treated with either the placebo emulsion or with the sample. The intensity of the redness of their skin was measured every five minutes by means of a Chromameter over a total period of 90 minutes. The changes in the measured and mean extinction values are listed in Table 1 and show a significantly lower intensity of redness for the sample in comparison to the placebo, whereby the intensity of redness also rapidly returns to the normal value.

Table 1

Time (minutes)	Extinction with the Placebo	Extinction with the Sample	Difference
5	7.20	6.90	0.30
10	6.98	6.82	0.16
15	7.04	6.93	0.11
20	7.06	6.47	0.59
25	6.55	5.71	0.84
30	6.03	5.39	0.64
35	4.91	4.56	0.65
40	4.58	3.99	0.56
45	3.65	3.10	0.55
50	3.82	2.47	0.81
55	2.85	2.22	0.63
60	2.38	1.83	0.55
65	1.96	1.40	0.56
70	2.15	1.29	0.86
75	1.74	1.00	0.74
80	1.25	0.93	0.32
90	1.29	1.29	0

Determination of the Elastase Inhibiting Activity of a Lima Bean Protein Fraction

500 µL of a solution of MeOSuc-Ala-Ala-Pro-Val-pNA, 1 mM, in an albumin containing buffer of pH 7.5 were mixed in each case with 10 µL of samples from an aqueous dilution series of the protein fraction, which had been manufactured in accordance with example 2, or with 10 µL of distilled water (reference), and then pre-incubated for 15 minutes at 37 °C. 100 µL of PMN elastase, 100 nM [sic; 100 mM?], were added to each test mixture, and the liberation of p-nitroaniline was registered over a period of 10 minutes at 405 nm by means of a recording photometer; the differences in absorption per minute (DA/min) were calculated therefrom and then, finally, the % elastase inhibition was calculated in accordance with the following equation:

Key to following equations:
% Hemmung = % Inhibition
Ref. = reference
Probe = sample
min. = minute

$$\% \text{ Hemmung} = \frac{DA_{Ref.}/\text{min} - DA_{Probe}/\text{min}}{DA_{Ref.}/\text{min}} \times 100$$

An I_{50} value (PMN elastase) of 89 mg/L (based on the dry substance) was determined for the protein fraction from a graphical dose/effect curve.

Example 6

Determination of the Trypsin Inhibiting Activity of an Inflammation Inhibiting Protein Fraction from Soybeans

0.100 mL of trypsin solution, 30 U [units]/mL, was, in each case, mixed with 0.100 mL of samples from an aqueous dilution series of the protein fraction that had been manufactured in accordance with example 1 or with 0.100 mL of distilled water (reference), and held at room temperature for one minute. 1.7 mL of tris-imidazole buffer of pH 8.4 and 0.100 mL of a 4 millimolar solution of Bz-Val-Gly-Arg-pNA was added to each test preparation at 37 °C, and the liberation of p-nitroaniline was registered over a period of 10 minutes by means of a recording photometer; the differences in absorption per minute (DA/min) were calculated therefrom and then, finally, the % trypsin inhibition was calculated in accordance with the following equation:

$$\% \text{ Hemmung} = \frac{DA_{Ref.}/\text{min} - DA_{Probe}/\text{min}}{DA_{Ref.}/\text{min}} \times 100$$

An I_{50} value of 3 mg/L (based on the dry substance) for the protein fraction was determined from a graphical dose/effect curve.

Example 7

Determination of the Tryptase Inhibiting Activity of an Inflammation Inhibiting Protein Fraction from Soybeans

125 µL of heparin, which had been dissolved in tris-HCl buffer of pH 7.6 (c= 10 mg/mL), and 125 µL of Tos-Gly-Pro-Arg-pNA,1.5 mM, were pipetted into 1000 µL of this buffer that contained different proportions by volume of the stable active substance concentrate that had been manufactured in accordance with example 3. 125 µL of human tryptase from the lung (c = 28 U [units] /mL) were added to each test mixture, and the liberation of p-nitroaniline was registered at 25 °C with a recording photometer over a period of 3 minutes at 405 nm; the differences in absorption per minute (DA/min) were calculated therefrom and then, finally, the % tryptase inhibition was calculated in accordance with the following equation:

$$\% \text{ Hemmung} = \frac{DA_{Ref.}/\text{min} - DA_{Probe}/\text{min}}{DA_{Ref.}/\text{min}} \times 100$$

An I_{50} value of 160 mg/mL for the active substance concentrate was determined from a graphical dose/effect curve.

Example 8

Action of an Inflammation Inhibiting Protein Fraction in an ex vivo Test System

A tendon, which had been isolated from the tail of a white mouse of the NMRI strain, was secured in an organ bath at room temperature that contained 0.5 mL of Tyrode solution at pH 7.4. The tendon was connected by means of a thread to a lever arm that converted any change in the length of the tendon into an electronic signal that was

amplified and registered using a potentiometer plotter. After twenty minutes of pre-incubation, the Tyrode solution was replaced by an adequate volume of Tyrode solution that contained, on the one hand, 38 µg of PMN elastase (= reference solution) and, on the other hand, 38 µg of PMN elastase along with 100 µL/mL of the active substance solution (= test solution) that had been manufactured in accordance with example 3. In the case of the reference solution, it was possible to register a continuous elongation of the tendon that subsequently tore after 132 minutes. No change in tendon length was observed in the case of the test solution, however.

Example 9

Determination of the Elastase Inhibiting Action of an Inflammation Inhibiting Protein Fraction in Fibroblast Cultures

Swiss 3T3 mouse fibroblasts with a cell density of 10^8 cells per 175 cm^2 were lyzed with 10 mL of 50 mM tris-HCl at pH 8.0 containing 1 % Triton X-l00 over a period of 30 minutes at 4 °C. The cell fragments were separated by centrifugation for 15 minutes at 5000 g and 0 °C. 500 µL of lysate were mixed with 500 µL of various concentrations of the active substance concentrate, which had been manufactured in accordance with example 3, and 34 µL of a solution of MeOSuc-Ala-Ala-Pro-Val-pNA and the liberation of p-nitroaniline was registered with a recording photometer over a period of 2 hours at 405 nm; the differences in absorption per minute (DA/min) were calculated therefrom and then, finally, the % elastase inhibition was calculated in accordance with the following equation:

$$\% \text{ Hemmung} = \frac{DA_{Ref./\,min} - DA_{Probe\,/\,min}}{DA_{Ref./\,min}} \times 100$$

An I_{50} value (fibroblast-elastase) of 280 mg/mL for the active substance concentrate was determined from a graphical dose/effect curve.

Example 10

Manufacture of a Cosmetic Creme (Oil-in-Water Emulsion)

Active substance concentrate in accordance with Example 3	10.0 g
Polysorbate 60	3.0 g
Sorbitan stearate	2.0 g
Cetyl alcohol	3.0 g
Stearic acid	6.0 g
Isopropyl myristate	10.0 g
Caprylic/capric acid triglycerides	5.0 g
Phenonip^R	0.5 g
Demineralized water	56.2 g
Propylene glycol	4.0 g
Imidazolinidyl urea [sic]	0.3 g

All the components, with the exception of the active substance concentrate, are mixed together at 70 °C with vigorous stirring; they are subsequently allowed to become cold and then they are mixed with the active substance concentrate.

Example 11

Manufacture of a Lotion for the Treatment of Inflammatory Diseases of the Skin

Active substance concentrate in accordance with example 3	5.0 g
Stearyl alcohol	1.0 g
Cetearate-6	1.0 g
Cetearyl alcohol	7.0 g
Mineral oil	8.0 g
Cetyl alcohol	1.0 g
Glyceryl stearate	2.5 g
PhenonipR	0.3 g
Demineralized water	72.0 g
Propylene glycol	2.0 g
Imidazolinidyl urea [sic]	0.2 g

All the components, with the exception of the active substance concentrate, are mixed together at 70 °C with vigorous stirring; they are then allowed to become cold and they are subsequently mixed with the active substance concentrate.

Example 12

Manufacture of a Skin Care Gel

Active substance concentrate in accordance with example 3	5.0 g
Demineralized water	88.5 g
Phenonip®	0.3 g
Imidazolinyl urea [sic]	0.2 g
Propylene glycol	5.0 g
Cellulose gum	1.0 g

All the components are dissolved in demineralized water at 40 °C with stirring.

Example 13

Action of a Soy Protein Fraction on the Moisture Content of the Skin

Over a test period of seven days, 60 µL of active substance concentrate in accordance with example 3 were in each case distributed over 20 cm^2 of the skin on the inner side of the left forearm of 5 test persons on five days; their untreated right arm served as the control in each case. The moisture of the skin, expressed in corneo units (CU), was measured two hours after the application, and the percentage increase in the moisture content of the skin was calculated from the measured data relative to the initial value. The results are illustrated in Table 2 in the form of the mean values from five test persons.

Table 2

Day	Corneo Units		Increase (%)	
	Sample	Control	Sample	Control
1	61.4	59.33	0	0
3	82.4	63.6	342	7.2
4	88.8	67	44.6	12.9
5	89.2	64.8	45.3	9.2
6	88	60.2	43.3	1.5
7	85	62.4	38.4	8.2

Example 14

Action of a soybean protein fraction on the elasticity of the skin

60 µL of the active substance concentrate in accordance with example 3 were applied twice daily to the corner of the left eye of 5 test persons; the untreated corner of their right eye served as the control in each case. The elasticity of the skin was determined prior to starting, as well as 14 days after the treatment, by measuring the depth of penetration of the skin into a hollow probe under negative pressure as well as the restoration of the skin under normal pressure. The difference in the depth of penetration of skin that had been stressed five times sequentially and then equilibrated again (= ds) is a measure of the elasticity of the skin. The smaller the difference, the greater the elasticity. The results are illustrated in Table 3 in the form of mean values from five test persons.

Table 3

	ds Left (Sample)	ds Right (Control)
Before Treatment	0.067 mm	0.057 mm
After Treatment	0.042 mm	0.058 mm

Patent Claims

1. Protein fraction that contains at least one protein, characterized by
   a) its isolation from leguminosae seeds,
   b) at least one band in the polyacrylamide gel electrophoresis procedure with sodium dodecylsulfate,
   c) molecular masses from 3,000 to 30,000 g/mole,
   d) a total nitrogen content of 14 % to 20 % and an amino-nitrogen content of 1 % to 2 % based on the protein content,
   e) its solubility in water and aqueous electrolyte solutions and its insolubility in ethanol and acetone,
   f) its marked precipitation in aqueous solution following the addition of trichloracetic acid, sulfosalicylic acid, picric acid or benzethonium chloride,
   g) its inhibition of proteinases

2. Protein fraction in accordance with claim 1, characterized by the feature that the leguminosae seeds are soybeans or lima beans.

3. Process for the isolation of the protein fraction in accordance with claim 1 or 2, characterized by the following steps:
   a) grinding the dried seeds,
   b) degreasing the flour by means of extraction with an organic solvent or solvent mixture,
   c) extracting the flour with water or an aqueous electrolyte solution at pH 2 to 10,
   d) evaporatively concentrating the extract at pH 5 to 7 and filtering or centrifuging the concentrate,
   e) separating the proteins, which are located therein, by means of precipitation,
   f) filtration or centrifugation and subsequent drying or lyophilization.

4. Stable aqueous active substance concentrate that contains 1 to 15 % by mass, and preferably 7.5 % by mass, based on the mass of the active substance concentrate, of a protein fraction in accordance with one of the claims 1 or 2 and at least one water soluble preserving agent and/or at least one polyhydric alcohol and/or a non ionogenic or amphoteric surfactant.

5. Active substance concentrate in accordance with claim 4 that contains methyl p-hydroxybenzoate as the water soluble preserving agent, and propylene glycol as the polyhydric alcohol, and polysorbate 80 and/or octoxynol and/or cocoamphoglycinate as the surfactant.

6. Use of a protein fraction in accordance with one of the claims 1 or 2 or of an active substance concentrate in accordance with one of the claims 4 or 5 for the manufacture of preparations for skin care and/or for the treatment of inflammatory diseases of the skin.

7. Preparations for skin care and/or for the treatment of inflammatory diseases of the skin, whereby these preparations contain a protein fraction in accordance with one of the claims 1 or 2 in a quantity, based on the total mass of the preparation, of 0.01 to 5 % by mass, and preferably 0.1 to 2 % by mass, in dry substance form.

8. Preparation, which has been applied to a plaster for transdermal application, whereby the preparation contains a protein fraction in accordance with one of the claims 1 or 2 in a quantity of up to 90 % by mass.

9. Process for the treatment of humans in the case of skin care and/or in the case of inflammatory diseases of the skin by applying an efficacious quantity of a protein fraction in accordance with one of the claims 1 or 2, an active substance concentrate in accordance with one of the claims 4 or 5, or a preparation in accordance with claim 7 or 8.

[LOGO] European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 92 81 0676

which, in accordance with Regulation 45 of the European Patent Agreement, can be considered to be the European Search Report for the additional proceedings

RELEVANT DOCUMENTS

Category	Document identification with an indication, if required, of the decisive portions	Pertains to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	US-A-2 794 800 (L. RIENKS ET AL.) - - -		C07K15/10 A61K37/64 A61K7/48 A61K9/70
A	EP-A-0 420 600 (CZECHOSLOVAK ACADEMY OF SCIENCES) - - - -		
			TECHNICAL AREAS SEARCHED (Int. Cl. 5) C07K A61K

INCOMPLETE SEARCH

In the opinion of the Search Department, the present European patent application complies with the regulations of the European Patent Agreement to such a small extent that, on the basis of some of the patent claims, it is not possible to carry out meaningful determinations in regard to the prior art.
Completely searched patent claims:
Incompletely searched patent claims:
Non searched patent claims:
Reason for the restriction of the search:

See Supplementary Page C

Search office	Date of completion of the search:	Examiner:
The Hague	09 DECEMBER 1992	G.L.E. REMPP

CATEGORY OF DOCUMENTS CITED

X: of special significance taken alone
Y: of special importance taken in combination with another publication of the same category
A: technological background
O: non-written disclosure
P: intermediate literature

T: theories or principles underlying the invention
E: colliding patent application
D: document cited in the patent application
L: document cited for other reasons

&: member of the same patent family, corresponding document

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Comment:

The search has been carried out even though claim 9 pertains to a process for the treatment of the human/animal body (Article 52(4) EPU [European Patent Agreement]) and is based on the stated actions of the compound/composition.